REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and in view of the reasons which follow.

Introduction

Applicants have amended claims 1, 6-8, 13, 15, 19, 20 and 23. The claim amendments are made either to correct clerical errors or to address concerns raised by the examiner as explained below. Applicants have deleted claims 11, 16 and 17, without prejudice or disclaimer. The amendments made herein are not intended to further limit the claims or acquiesce to the propriety of the examiner's position in any rejection. The amendments made herein are introduced to advance the case towards allowance.

Claim Rejection – Non Art Related Remarks

35 U.S.C. §112, second paragraph

The examiner has rejected claims 1-23 as allegedly indefinite for various reasons. Applicants respectfully traverse these rejections and address each rejection below.

1. Claims 1, 6 and 23 are rejected for the language "an encoding sequence" in step (a) because it is allegedly unclear whether the claimed sequence codes for "human coagulation factor VIII" or not. In order to address the examiner's concern, applicants have amended claims 1, 6 and 23 to read "a sequence encoding human coagulation factor VIII."

2. Claims 1, 8, 13, 19, 20 and 23 are rejected for the lack of antecedent basis. Applicants have amended claims 1, 8, 13, 19, 20 and 23 to provide sufficient antecedent basis for the objected phrases.

3. With respect to the objection to the recitation of "additional regulatory element encoding a signal peptide" as allegedly indefinite, applicants have amended

claim 20 to delete "additional regulatory element" and to read "a sequence encoding a signal peptide."

4 In claim 6 the examiner objects to the language "genetic material encoding the production of the human coagulation factor VIII" in step (b). Applicants have amended claim 6 to delete the language "the production of."

5. Claim 7 is rejected as allegedly indefinite for reciting "genomic DNA" because it is unclear how to transform and express an entire genome in a transgenic plant, and further for the language "combinations thereof." While not acquiescing to the propriety of the examiner's rejection, applicants have obviated the rejection by removing the rejected terms "genomic DNA" and "combinations thereof."

6. Claim 11 is rejected as allegedly indefinite for the language "substantially that of human coagulation factor VIII." Without acquiescing to the propriety of the examiner's position, applicants have obviated the rejection by canceling claim 11.

7. Claim 15 is rejected as allegedly indefinite because it is unclear how a human/porcine factor VIII would still be considered as a "human" protein. Applicants have amended claim 15 to address the examiner's concern.

8. Claims 16 and 17 are rejected as allegedly indefinite because it is unclear whether the claimed heavy and light chain proteins are still human, as in claim 6. Without acquiescing to the propriety of the examiner's rejection, applicants have obviated this rejection by canceling these claims.

Applicants respectfully submit that the above amendments to the claims fully address the examiner's concerns, and thus render the rejection moot. Accordingly, withdrawal of the rejection is respectfully requested.

35 U.S.C. §112, first paragraph

Claims 1-23 are rejected under 35 U.S.C. §112, first paragraph, as allegedly non-enabled. Applicants respectfully traverse the rejection.

At the outset, with respect to the rejection of claim 7, applicants note that claim 7 has been amended to delete the recitation of genomic DNA, which renders the rejection moot.

The examiner contends that claims 1-23 are not enabled for a method of expression of ***any*** encoding sequence having ***any*** modification from ***any*** vector in ***any*** plant, while enabling for methods of expression of a known human or porcine/human chimera coagulation factor VIII in tobacco plants. The examiners appears to assume the following factors as unpredictable or are of unreasonable scope:

(a) the number of sequences claimed, *i.e.,* any sequence including full length, B-domain deletions, VIIIa, porcine/human recombinants, and heavy and light chains;

(b) the number of modifications in the nucleotide sequences used to produce human coagulation factor VIII in transgenic plants; and

(c) the use of any plant species for production of human coagulation factor VIII.

The examiner clearly fails to appreciate applicants' significant achievement and contribution to this important scientific field in providing the first successful production of large and complex protein, a human coagulation factor VIII, in a transgenic plant. In fact, a human coagulation factor VIII is by far the largest single frame protein ever expressed in a transgenic plant.

Applicants' specification is presumed to be enabling, absent objective evidence to the contrary. Thus, the examiner bears the initial burden of proffering either objective evidence or sound scientific reasoning to explain why the claims allegedly are not supported by an enabling disclosure. The examiner, however, has failed to meet this burden for the reasons set forth below.

The standard for determining enablement is whether the specification as filed provides sufficient information as to permit one skilled in the art to make and use the claimed invention. United States v. Telectronics, Inc., 8 USPQ2d 1217, 1223 (Fed. Cir. 1988). The test of enablement is not whether experimentation is necessary, but rather

whether any experimentation that is necessary is undue. In re Wands, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). Thus, neither the breadth of claims nor the absence of working examples is a dispositive factor. Similarly, enablement does not require actual reduction to practice. In addition, since only an enabling disclosure is required, applicant needs not describe all actual embodiments. MPEP § 2164.02 (Rev. Feb 1, 2000). Moreover, it is "not a function of the claims to specifically exclude inoperative substances." Atlas Powder Co. v. E.I. du Pont de Nemours & Co., 750 F.2d 1569 (Fed. Cir. 1984). The question is not whether all embodiments are operative, but whether one of skill in the art could determine which ones are operative.

Nucleotide sequences coding for human coagulation factor VIII, its functional domains including domains A, B and C, and the occurrence of these domains in heavy and light chains, were already known in the art as described in the specification (page 14, lines 11-16) and as disclosed in more detail in the publications, which are incorporated by reference in the specification at page 2, lines 22-25. These publications include Wood *et al.*, Expression of active human factor VIII from recombinant DNA clones, *Nature*, vol 312, pp 330-337 (1984) and Kaufman *et al.* Synthesis, processing, and secretion of recombinant human factor VIII expressed in mammalian cells, *The J. of biological chemistry*, vol 263, No. 13, pp 6352-6362 (1988). Further, the accompanying reference, Pittman *et al.*, Biochemical, Immunological, and *In vivo* functional characterization of B-domain-deleted factor VIII, *Blood*, vol 81, no 11 pp 2925-2035, (1993) discloses the production and characterization of a B-domain-deleted from of the factor VIII, and shows that it has biochemical, immunologic and *in vivo* functional properties that are very similar to wildtype human factor VIII. Contrary to the examiner's assertion, numerous nucleotide sequences coding for functional human factor VIII protein, or functional fragments of human factor VIII protein, were already identified at the time of filing of the instant application. Thus, it would not have required undue experimentation at the time of filing of the instant application to identify nucleotide sequences coding for human factor VIII protein, or functional fragments of human factor VIII protein, for expression in transgenic plants. Nonetheless, in order to expedite prosecution of the case, applicants have cancelled claims 16 and 17, which recite sequences coding for heavy and light chains, respectively.

Similarly, further modifications in the human factor VIII coding sequence were readily accomplished by one skilled in the art at the time of filing of the instant application. The specification clearly teaches that "modifying encoding sequence is accomplished using standard molecular cloning procedures as described in Ausubel et al. 1992, Current protocols in molecular biology, Wiley, New York." Furthermore, the specification specifically describes how to modify encoding sequences, for example adding a transcription promoter and transcription terminator. See page 13, lines 1-14.

The examiner appears to rely on the Cramer *et al.* ("Cramer") reference to provide evidence that the disclosure is not enabling for the claimed method in any plants. The examiner contends that it would not have been predictable to express any protein in any plant species. Cramer merely refers to tobacco as the easiest plant to genetically engineer. However, Cramer does not provide any insight into whether protein production in other plants is unpredictable. The examiner fails to provide any evidence to support his conclusion that protein production using plants other than tobacco is unpredictable and that the production of human factor VIII from other plants requires undue experimentation.

As the examiner admits, the specification describes production of human factor VIII using the full-length polypeptide of human factor VIII cDNA from tobacco in Example 1. As discussed above, numerous other nucleotide sequences coding for human factor VIII were known in the art at the time of filing of the instant application, or could have been readily ascertained using the teachings of the instant application.

Accordingly, applicants respectfully submit that, by following the disclosure in the specification, combined with the well-known technology, one skilled in the art could perform the instantly claimed methods without undue experimentation.

Therefore, because the instant claims 1-23 meet the standard of 35 U.S.C. §112, first paragraph, applicants respectfully request that the examiner withdraw the rejection.

Claim Rejection – Art Related Remarks

Claims 1-23 are rejected under 35 U.S.C. §103(a) as allegedly obvious over Cramer, in view of Hoeben et al., Healey et al., Lollar et al., Stein et al., and Lubin et al.

Cramer discloses production of human enzyme C from a transgenic tobacco. However, Cramer does not specifically teach or suggest the production of human coagulation factor VIII in transgenic tobacco plants. Moreover, although Cramer enumerates various proteins that have been successfully expressed in transgenic plants or cultured plant cells, such as human serum albumin, human alpha interferon, human erythropoietin, leu-enkephalin, rabbit liver cytochrome P-450, hamster 3-hybroxy-3-methylglutaryl CoA reductase, and murine IgG and IgA immunoglobulins, there is no mention as to the production of human factor VIII in a transgenic plant.

As indicated in Cramer, production of human proteins, such as glycoproteins, requires complex processing steps including post-translational modification to provide the protein in an active form. See page 64, lines 1-2. For example, Cramer discloses that γ-carboxylation of the amino terminal "GLA" domain, which is catalyzed a vitamin K-dependent gamma-glutamyl carboxylzase, is critical for activation of plant-derived hPC. See page 65, lines 1-4. A γ-carboxylase activity has not been identified in plants. Furthermore, Cramer failed to test whether tobacco-derived hPC has biological activity. See page 65, lines 1-2 of the second full paragraph. Indeed, if tested, recombinant plant-derived hPC would not be active because the processing of the "GLA" domain cannot occur *in planta*.

Cramer suggests that "correct processing of the hPC GLA domain may require co-expression of the human γ-carboxylase cDNA in tobacco." See page 64, line 1 from the bottom to page 65, line 6. However, successful γ-carboxylation of complex GLA-proteins such as hPC requires the presence of the Vitamin K cycle in the organism responsible for post-translational processing (Berkner, *J. Nutr* 130:1877, 2000). The mere presence of γ-glutamyl carboxylase is not sufficient for conversion of glutamic acid to γ-carboxyglutamic acid as a regenerating supply of Vitamin KH_2 (hydroquinone) cofactor is necessary for function of the carboxylase enzyme (Van Cott et al. *Genet Anal.* 15:155, 1999). In order to successfully facilitate this post-translational

modification in any transgenic organism, either a continuous, exogenous supply of the hydroquinone must be provided or the remainder of the vitamin K cycle, including processing enzymes for the conversion of vitamin K epoxide to vitamin K and subsequently to vitamin KH_2, must be expressed either by native conversion enzymes or through further genetic modification of the organism. Thus γ-carboxylation *in planta* would require either expression of at least three separate enzymes to facilitate an active vitamin K cycle or exogenous feeding of vitamin KH_2 in addition to γ-glutamyl carboxylase expression. Cramer, therefore, fails to teach or to obtain bioactive hPC in a transgenic plant.

Cramer admits that "[o]ur results with tobacco-based production of human protein C demonstrated the capabilities of plant cells to synthesize and partially process this complex serum protease. However, this work also highlighted the limitations imposed by low levels of transgene expression and the need to develop plant strains conferring specialized protein-processing capabilities." See page 68, lines 5-10 of Cramer

In order for the examiner to properly rely on a reference, it must be enabling. Rockwell Int'l Corp v. United States, 147 F.3d 1358 (Fed. Cir. 1998), Motorola, Inc. v. Interdigital Tech. Corp., 121 F.3d 1461, 1471 (Fed. Cir. 1997) ("In order to render a claimed apparatus or method obvious, the prior art must enable one skilled in the art to make and use the apparatus or method." (quoting Beckman Instruments, Inc. v. LKB Produkter AB, 892 F.2d 1547, 1551 (Fed. Cir. 1989)). Considering the totality of the Cramer reference, it highlights the apparent limitations in producing highly processed, complex, human proteins, such as the human factor VIII, in transgenic plants.

In contrast, in the instant application, the appearance of comparable immunoreactive bands in both the plant-derived and plasma-derived human factor VIII, shows that the human factor VIII produced by the claimed methods undergoes correct, human-like post-translational modifications *in planta*. See page 15, lines 17-19. The accompanying declaration prepared by Dr. Brian C. Hooker, one of the co-inventors, confirms that the claimed method produce a bioactive human factor VIII by testing the bioactivity of the human factor VIII produced by the claimed method.

Hoeben et al., Healey et al., Lollar et al., Stein et al., and Lubin et al. are cited as teaching human and/or human/porcine chimeras of coagulation factor VIII. Further, the examiner cites Hoeben et al alleging that it teaches motivation for recombinant expression of human coagulation factor VIII for use in the treatment of hemophilia. None of the cited references, however, cure the deficiencies in the Cramer publication discussed above.

Hoeben et al. teach a retroviral vector system for the transfer of factor VIII cDNA expression vectors into murine fibroblast cell lines and primary human skin fibroblasts in tissue for gene therapy. However, there is no indication in Hoeben et al. that a bioactive human factor VIII can be produced in transgenic plants. Nothing in Hoeben et al. teaches or suggests that recombinant production of bioactive human factor VIII can be successfully carried out using plants.

None of the cited references provide motivation for producing bioactive human factor VIII in transgenic plants. None of the cited references provide motivation to express any protein that is subject to extensive post-translational modifications in transgenic plants. Cramer clearly highlights the obstacles of producing proteins that are subject to post-translation modifications in transgenic plants and therefore teaches away from the solution claimed in the instant application. None of the secondary references, including Hoeben et al., provides the motivation that is clearly lacking in Cramer.

Since one of ordinary skill in the art would not have had a reasonable expectation of success of producing bioactive human factor VIII based on the teachings of the prior art cited by the examiner, there cannot be a *prima facie* case of obviousness.

Assuming, *arguendo*, if the examiner has established *prima facie* case of obviousness, objective evidence of unexpected results rebuts such a case. Expression of bioactive human factor VIII *in planta* was unexpected in view of the failure of the prior art, such as Cramer, to produce a bioactive protein *in planta*.

The attainment of unexpected results or properties is a powerful demonstrations of patentability. See U.S. v. Adams, 383 U.S. 39, 51-52 (1966); Lindemannn Maschinenfabrik v. American Hoist and Derrick Co., 730 F.2d 1452, 1461 (Fed. Cir.

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Accordingly, withdrawal of the rejection is respectfully requested.

In view of the foregoing amendment and remarks, applicant respectfully requests favorable reconsideration and allowance of the pending claims. If there are any issues remaining which the examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the examiner hereby respectfully invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

Date April 2, 2002

By _____

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Marked up rewritten claims:

1. (Amended) A method of producing an active human coagulation factor VIII, comprising:

(a) subcloning [an encoding sequence] a sequence encoding human coagulation factor VIII into a plant expression vector and obtaining a subcloned plant expression vector;

(b) transferring the subcloned plant expression vector into a plurality of plant cells;

(c) selecting a plurality of positive transformants from the plurality of plant cells on an antibiotic selective media;

(d) growing the plurality of plant cells in whole plants or suspensions; and

(e) extracting and purifying the human coagulation factor VIII from the plurality of [transgenic] plant cells.

6. (Amended) A method of producing [a] an active human coagulation factor VIII from plant cells, comprising the steps of:

(a) introducing [an encoding sequence] a sequence encoding human coagulation factor VIII for production of human coagulation factor VIII into a plant expression vector in the plant cells;

(b) obtaining a positive transformant of the plant cells, the positive transformant carrying genetic material encoding [the production of] the human coagulation factor VIII;

(c) cultivating the positive transformant; and

(d) obtaining the human coagulation factor VIII.

(b) transferring the subcloned plant expression vector into a plurality of plant cells;

(c) selecting a plurality of positive transformants from the plurality of plant cells on an antibiotic selective media;

(d) growing the plurality of plant cells in whole plants or suspensions; and

(e) extracting and purifying the human coagulation factor VIII from the plurality of [transgenic] plant cells.

7. (Amended) The method as recited in claim 6, wherein said encoding sequence is a cDNA [in from the group consisting of copy DNA, genomic DNA and combinations thereof].

8. (Amended) The method as recited in claim 6, wherein factor VIII is cultivated in [the] a whole plant.

13. (Amended) The method as recited in claim 6, wherein said encoding sequence encodes a full length of said human coagulation factor VIII deleting [the] a B-domain.

15. (Amended) The method as recited in claim 6, wherein a sequence encoding A2 epitope of human coagulation factor VIII in said sequence is replaced with an analogous porcine sequence [said encoding sequence encodes human/porcine factor VIII wherein is replaced with an analogous sequence in the A2 epitope of human factor VIII is replaced with the analogous porcine sequence].

19. (Amended) The method as recited in claim 6, wherein said encoding sequence is provided by adding [said] a transcription promoter to the upstream of 5′ end of the encoding sequence; and adding [said] a transcription terminator to the downstream of 3′ end of the encoding sequence.

20. (Amended) The method as recited in claim 19, further comprising adding [an additional regulatory element] a sequence encoding a signal peptide [, said

additional regulatory element added] between the transcription promoter and the upstream 5′ end of the encoding sequence.

23. (Amended) A method of producing [a] an active human coagulation factor VIII using an agrobacterium mediated transformation, comprising:

(a) modifying a coagulation factor VIII [encoding sequence] a sequence encoding human coagulation factor VIII for subcloning into a plant expression vector;

(b) subcloning the encoding sequence into the plant expression vector;

(c) transferring the plant expression vector to agrobacterium;

(d) co-cultivating a portion of the transgenic plant cells with the agrobacterium;

(e) selecting positive transformants from the co-cultivated culture on an antibiotic selective media;

(f) permitting growth of [the] transgenic plant cells in whole plants or suspensions; and

(g) extracting a quantity of human coagulation factor VIII from the plant cells.